

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



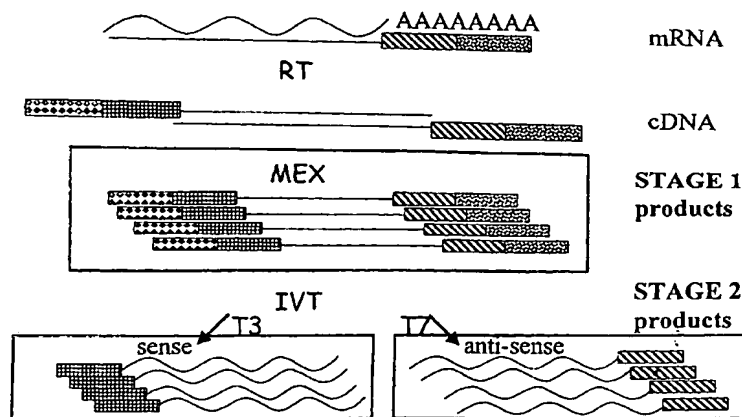
(43) International Publication Date
22 January 2004 (22.01.2004)

PCT

(10) International Publication Number
WO 2004/007766 A1

- (51) International Patent Classification⁷: C12Q 1/68, C12N 15/10
- (74) Agent: GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).
- (21) International Application Number: PCT/GB2003/002989
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 10 July 2003 (10.07.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 0216026.5 10 July 2002 (10.07.2002) GB
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): CAMBRIDGE BIOTECHNOLOGY LTD. [GB/GB]; 10 Willington Street, Cambridge CB1 1HW (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): DIXON, Alastair [GB/GB]; Cambridge Biotechnology Ltd., 10 Willington Street, Cambridge CB1 1HW (GB). SKYNNER, Michael [GB/GB]; Cambridge Biotechnology Ltd., 10 Willington Street, Cambridge CB1 1HW (GB).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: NUCLEIC ACID AMPLIFICATION METHOD



(57) **Abstract:** A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, comprises the steps of: (i) reverse transcription of the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each primer sequence is unique, and either or each heel sequence includes a RNA polymerase promoter site, and the FAP includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence; and (ii) amplification of the cDNA using primers sufficiently complementary to the primers, i.e. FAP and TAP, within FAP-RAND and TAP-RT. In one embodiment, the method additionally comprises the step of: (iii) *in vitro* transcription, to produce RNA run-offs from either end of the amplicons.